device that is placed adjacent to the microfluidic device being utilized to conduct a thermal cycling reaction such that the laser light from the diode is directed into the detection section.

[0041] Detectors can be microfabricated within the microfluidic device, or can be a separate element. A number of commercially-available external detectors can be utilized. Many of these are fluorescent detectors because of the ease in preparing fluorescently labeled reagents. Specific examples of detectors that are available include, but are not limited to, Applied Precision ArrayWoRx (Applied Precision, Issaquah, Wash.) and the ABI 7700 (Applied Biosystems, Inc., Foster City, Calif.).

Fabrication

[0042] Microfluidic devices are generally constructed utilizing single and multilayer soft lithography (MSL) techniques and/or sacrificial-layer encapsulation methods. The basic MSL approach involves casting a series of elastomeric layers on a micro-machined mold, removing the layers from the mold and then fusing the layers together. In the sacrificial-layer encapsulation approach, patterns of photoresist are deposited wherever a channel is desired. These techniques and their use in producing microfluidic devices is discussed in detail, for example, by Unger et al., 2000, *Science* 288:113-116; by Chou, et al., 2000, "Integrated Elastomer Fluidic Lab-on-a-chip-Surface Patterning and DNA Diagnostics, in Proceedings of the Solid State Actuator and Sensor Workshop, Hilton Head, S.C.; in PCT Publication WO 01/01025; and in published U.S. patent application No. 20050072946 (each incorporated herein by reference).

[0043] In one approach, the foregoing fabrication methods initially involve fabricating mother molds for top layers (e.g., the elastomeric layer with the control channels) and bottom layers (e.g., the elastomeric layer with the flow channels) on silicon wafers by photolithography with photoresist (Shipley SJR 5740). Channel heights can be controlled precisely by the spin coating rate. Photoresist channels are formed by exposing the photoresist to UV light followed by development. Heat reflow process and protection treatment is typically achieved as described by Unger et al. supra. A mixed two-part-silicone elastomer (GE RTV 615) is then spun into the bottom mold and poured onto the top mold, respectively. Spin coating can be utilized to control the thickness of bottom polymeric fluid layer. The partially cured top layer is peeled off from its mold after baking in the oven at 80° C. for 25 minutes, aligned and assembled with the bottom layer. A 1.5-hour final bake at 80° C. is used to bind these two layers irreversibly. Once peeled off from the bottom silicon mother mold, this RTV device is typically treated with HCL (0.1N, 30 min at 80° C.). This treatment acts to cleave some of the Si—O—Si bonds, thereby exposing hydroxy groups that make the channels more hydrophilic.

[0044] The device can then optionally be hermetically sealed to a support. The support can be manufactured of essentially any material, although the surface should be flat to ensure a good seal, as the seal formed is primarily due to adhesive forces. Examples of suitable supports include glass, plastics and the like.

[0045] In certain devices, the devices formed according to the foregoing method result in the substrate (e.g., glass slide) forming one wall of the flow channel. Alternatively, the device once removed from the mother mold is sealed to a thin elastomeric membrane such that the flow channel is totally enclosed in elastomeric material. For certain uses, e.g., PCR amplification, flow channels and chambers enclosed in elastomeric material (i.e., without a glass wall) are preferred. The resulting elastomeric device can then optionally be joined to a substrate support. In some cases, the device is made as described in U.S. patent publication No. 20050072946. In some cases, the device uses "push-up valves" described in U.S. patent publication No. 20050072946 (e.g., FIG. **37**B). "Push-up" refers to low actuation pressure geometry in which the membrane deflects upwards to seal off the upper fluid channel. In this geometry, the deflectable membrane is featureless and exhibits a substantially constant thickness.

[0046] Reagents can be deposited in reaction chambers before addition of a sample to the MPD. A number of commercially available reagent spotters and established spotting techniques can be used to deposit the reagent(s). Microfluidic devices in which reagents are deposited at the reaction sites during manufacture are typically formed of three layers. The bottom layer is the layer upon which reagents are deposited. The bottom layer can be formed from various elastomeric materials as described in the references cited above on MLS methods. Typically, the material is polydimethylsiloxane (PDMS) elastomer. Based upon the arrangement and location of the reaction sites that is desired for the particular device, one can determine the locations on the bottom layer at which the appropriate reagents should be spotted. Because PDMS is hydrophobic, the deposited aqueous spot shrinks to form a very small spot. The deposited reagents are deposited such that a covalent bond is not formed between the reagent and the surface of the elastomer because, as described earlier, the reagents are intended to dissolve in the sample solution once it is introduced into the reaction site. In some versions, the reagent is designed to be inactive or unavailable to a reaction until a specified condition occurs (e.g., a polymerase not activated until heated or until the addition of a necessary cofactor).

[0047] The other two layers of the device are the layer in which the flow channels are formed and the layer in which the control and optionally guard channels are formed. These two layers are prepared according to the general methods set forth earlier in this section. The resulting two layer structure is then placed on top of the first layer onto which the reagents have been deposited. A specific example of the composition of the three layers is as follows (ration of component A to component B): first layer (sample layer) 30:1 (by weight); second layer (flow channel layer) 30:1; and third layer (control layer) 4:1. It is anticipated, however, that other compositions and ratios of the elastomeric components can be utilized as well. During this process, the reaction sites are aligned with the deposited reagents such that the reagents are positioned within the appropriate reaction site.

C. Partitioning, Detection and Analysis of Nucleic Acids

[0048] In this section, methods for analysis of nucleic acids in a sample are described. The methods involve massive partitioning of the sample and any nucleic acid molecules it contains, and amplification (as defined herein) of target sequences in the partitioned nucleic acid molecules. Various versions of the methods may also involve application of particular amplication strategies, pooling of amplification products, analysis of pooled amplification products and/or other features that will be apparent upon reading this disclosure.